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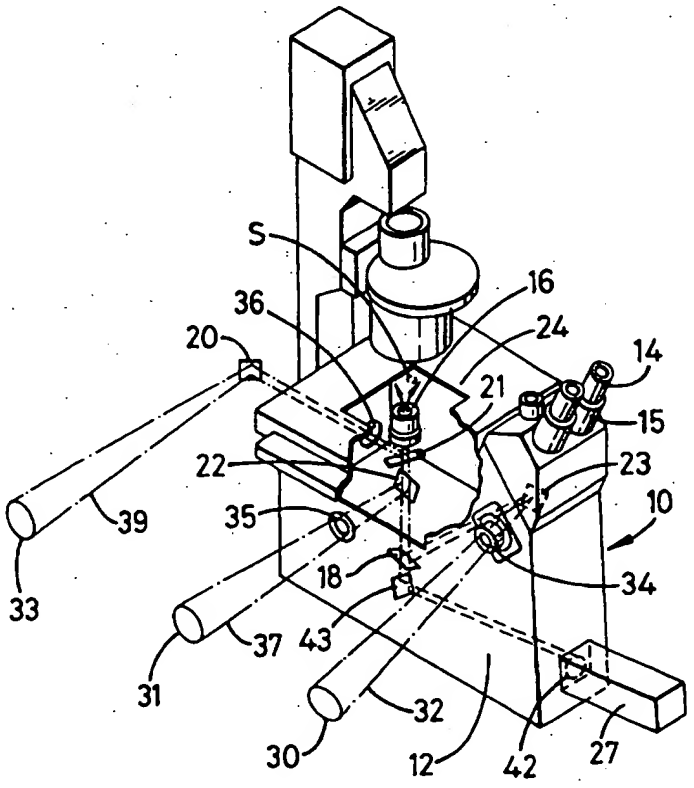
(54) Title: MICROSCOPE WITH LASER PORT

(57) Abstract

A microscope has been invented having a hollow base, viewing means, an objective lens assembly, a stage for holding a sample to be studied by the microscope, and at least one port in the base through which at least one laser beam is directable to manipulate (move, ablate, cut) a sample positioned on the stage. In one aspect such a microscope is provided with at least one laser for providing the at least one laser beam, the laser(s) mounted outside or inside the base. In one aspect the at least one laser is a diode laser which may be mounted in an interchangeable objective lens assembly. In one aspect the interchangeable lens assembly has at least one laser beam port and/or at least one laser mounted therein.



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MICROSCOPE WITH LASER PORT

BACKGROUND OF THE INVENTION

Field Of The Invention

This invention is related to microscopes, and in one aspect, to a microscope with an objective lens assembly that provides an entry port for a laser beam to be directed at microscopic samples under inspection. In one aspect a microscope with such a laser beam is useful for manipulating microscopic objects such as biological cells and microstructures.

Description of Related Art

Certain prior art microscopes have various windows, or ports, in their bases. These ports have various optical elements, such as lenses and mirrors, which direct light and images between the external environment and a sample on the microscope stage. These ports are used to introduce, e.g., ultraviolet light to stimulate fluorescence in the sample, or to provide videographic recordings of the sample. The physical structure of a microscope limits the number of ports that can be provided. In addition, it is difficult, often impossible, to add new ports to a given microscope design. Certain conventional ports involve the use of one or more fixed optical structures which are not easily modified after original manufacture of the microscope. These structures may be made of materials which are not suitable for use with lasers of useful wavelengths.

U.S. Patent 5,349,468 to Rathbone, et al, describes a microscope assembly containing a wavelength-selective

dichroic mirror situated in a microscope objective for directing externally produced light into a microscopic sample for the purpose of stimulating fluorescence in the sample.

External lasers may be used as microscopy tools, e.g. for optical trapping and laser microablation. Manipulation of microscopic structures is discussed in U.S. Patent Nos. 4,893,886; 4,887,721; 5,170,890; 5,212,382; 5,308,976; 5,363,190; 5,366,559; 5,367,160; 5,359,615; and 5,393,957. In general these patents discuss methods for trapping microscopic particles in a photon gradient, or for controllably damaging microscopic structures with laser energy.

The use of laser beams for the purpose of performing optical trapping, optical microdissection and laser-assisted molecular studies has been extensively described in the literature. These applications pertain to various fields of research and describe not only the manipulation of biological particles but also of non-biological samples such as plastic microparticles and crystals. The majority of the examples report the use of prototype installations. A smaller number of examples use commercially available optical trapping or optical microdissection apparatus. Prototype installations often apply a unique scheme of association of a laser module to a microscope via a large optical table. These installations are not always described in detail in the literature and may vary singularly from one another. Current commercially available optical trapping and optical microdissection apparatus present a more standardised means to interface the laser module to a microscope, yet without offering extensive controls over laser beam spatial characteristics. In the field of laser-assisted molecular studies, a number of laser-based technologies are already available that include elaborate

systems and mechanisms for the purpose of achieving laser scanning microscopy or confocal microscopy for example.

SUMMARY OF THE PRESENT INVENTION

The present invention, discloses, in certain embodiments, a microscope with a base, a mirror assembly and an objective lens assembly. A laser provides a laser beam which passes through a laser input port of the objective lens assembly. A wavelength selective mirror is also positioned inside the objective lens assembly at an appropriate angle (e.g. 45 degrees) so that the laser beam directed onto the mirror is reflected through the objective lens assembly and focused upon the sample for manipulation thereof (e.g. moving, ablating, and/or cutting). The geometry of the objective lens and the selective mirror allows useful laser light of the appropriate wavelength (e.g. in certain preferred embodiments from 100 nanometers to 5000 nanometers) to be projected through the objective lens assembly and into the sample, without interfering with visible light travelling in the conventional directions required for microscopic observation. Externally produced laser beams (e.g. useful for controlled ablation or trapping) may then be directed through such modified objective lens assembly without otherwise interfering with the use of the microscope. Alternatively, one or more lasers may be located in a base of the microscope or disposed on part of the microscope. A camera (still or video) may be located in the base or on part of the microscope. In one aspect a stage for a sample to be studied is manually movable for precise positioning relative to the objective lens. Alternatively a motor-driven stage may be used.

The present invention, in certain embodiments, discloses a microscope with a hollow base, an eyepiece,

an objective lens assembly, a stage for holding a sample to be studied by the microscope, and at least one port in the base through which at least one laser beam is directable to manipulate a sample positioned on the stage; such a microscope is combined with at least one laser for providing the at least one laser beam; such a microscope wherein the at least one laser is mounted exterior to the base of the microscope; such a microscope wherein the at least one laser is mounted within the base; such a microscope wherein the at least one laser is a diode laser; such a microscope wherein the at least one laser is a plurality of lasers; such a microscope wherein the objective lens assembly is an interchangeable assembly; such a microscope wherein the interchangeable lens assembly has at least one laser beam port; such a microscope wherein the interchangeable assembly has at least one laser mounted therein; such a microscope with at least one wavelength selective mirror on which the at least one laser beam is directed; such a microscope wherein the wavelength selective mirror permits simultaneous illumination of a sample on the stage and laser irradiation of the sample; such a microscope wherein the wavelength selective mirror permits visual observation of the sample and photographic recording of the sample; such a microscope wherein the stage is movably connected to the base; such a microscope with a motorised assembly connected to the stage for selectively and controllably moving the stage with respect to the base and with respect to the objective lens assembly; such a microscope with at least one barrier filter for filtering the at least one laser beam to exclude light of selective wavelengths from the at least one laser beam; such a microscope wherein the at least one laser is at least two lasers, the at least one laser beam is at least two laser beams, and the microscope has a barrier filter

for each of the at least two laser beams; such a microscope wherein the at least one wavelength selective mirror is a plurality of wavelength selective mirrors which provide the simultaneous transmission and reflection of selected wavelengths of laser light; and such a microscope wherein the laser light is from the group consisting of ultraviolet, visible and infrared.

It is, therefore, an object of at least certain preferred embodiments of the present invention to provide:

New, useful, unique, efficient, non-obvious microscopes with one or more laser beam input ports; and such a microscope (upright or inverted) in combination with one or more lasers (within or outside a base) and/or with a camera;

Such a microscope with one or more wavelength selective mirrors;

Such a microscope with one or more lasers for providing a laser beam and, in one aspect, one or more diode lasers;

Such a microscope in combination with a still camera and/or video camera;

Such a microscope with an interchangeable objective lens assembly and such an assembly with a laser input cylinder through which laser light is provided to the microscope;

Such a laser input cylinder with one or more laser diodes therein for providing laser light to the microscope; and

Such a microscope with one or more light sources (e.g. wavelength specific light) directed into a microscopic sample to illuminate it or stimulate fluorescence therein.

DESCRIPTION OF THE DRAWINGS

A more particular description of embodiments of the invention briefly summarised above may be had by references to the embodiments which are shown in the drawings which form a part of this specification:

Fig. 1 is a perspective view of a system according to the present invention.

Fig. 2A is a perspective view, partially cut-away, of a laser input assembly according to the present invention. Fig. 2B is a view of another side of the assembly of Fig. 1A.

Figs. 3 - 5, 6a-6c, and 7-9 show schematically laser beam steering mechanisms according to the present invention and parts thereof.

Fig. 3 shows a laser beam positioning component according to the present invention with circular wedge prisms shown in cross-section.

Fig. 4 shows a top view of a laser beam alignment component according to the present invention.

Fig. 5 shows a top view of a parfocality adjustment component according to the present invention.

Figs. 6a - 6c illustrate operation for the parfocality adjustment component of Fig. 5.

Fig. 7 shows a top view of an X-Y laser control component according to the present invention.

Fig. 8 illustrates operation for the X-Y laser control component of Fig. 7.

Fig. 9 shows a top view of a laser beam steering mechanism according to the present invention.

DESCRIPTION OF PREFERRED EMBODIMENTS

Fig. 1 illustrates schematically an inverted microscope 10 according to the present invention with a base 12, viewing binocular eyepiece 14, objective lens assembly 16, mirror 18, mirror 20, mirror 22, mirror 23, stage for sample mounting 24, and one or more lasers 30, 31, 33 (shown schematically) and laser beam 32, 37 and 39.

In one embodiment the laser 30 is an external laser light source which produces infrared light in the wavelength range between 0.750 micrometers (750 nanometers) and 5.0 micrometers (5000 nanometers). The laser beam produced by such a laser is projected into a hole or port 34 in the base 12 where it impinges upon wavelength selective mirrors 18 and 23 which are positioned at an angle complementary to the incidence angle (with respect to the mirror) of the incoming laser beam such that the laser beam is projected through the objective lens assembly 16 toward the microscope stage 24 and sample S (shown in dotted line, Fig. 1). Additional ports 35 and 36 in and through the base 12 are associated with additional wavelength-selective mirrors 20, 21, and 22 respectively. The multiple laser beams 32, 37 and 39 may originate from more than one laser or may originate from a common laser with the beam appropriately split to form more than one incident beam. In one aspect the wavelength selective mirrors select laser light with wavelengths between: 100 to 400 nanometers; 400 to 750 nanometers; and 750 to 5000 nanometers. For optimum selective light transmission mirrors coated with dichroic material are used to reflect specific ranges of wavelengths of light while other ranges of wavelengths of light are transmitted through the mirror. When light must only be reflected by a mirror (e.g. when the mirror

need not be transparent to some selective wavelengths of light), then gold-coated mirrors may be used for optimum IR reflection (e.g. from 750 nm through 5000 nm) and enhanced aluminium mirrors for optimum reflection of visible light (e.g. 400 nm through 750 nm), or multilayer reflectors centred at a specific wavelength may be used for optimum UV reflection (e.g. between 100 nm and 400 nm).

As is common in the art a sample S may be in a dish, on a slide, or in some other container which is placed on the stage or the stage may include a glass or other transparent plate on which the sample is placed for viewing. A camera 27 (shown schematically, Fig. 1) secured to the base 12 (although it may be positioned adjacent the base 12 without being secured thereto) receives a light beam B from the sample S reflected by a mirror 43 through a port 42 in the base. The camera 27 may be a still photography camera or a video camera.

In one aspect the mirror 23 is made of dichroic materials or dichroic coated mirrors such that the mirrors selectively reflect light which is of a wavelength above 0.750 micrometers and which intersects the surface of the mirror at an angle above 0.0 degrees when such angle is measured relative to an imaginary line orthogonal to the surface of the mirror. Preferably light in the spectrum below 0.750 micrometers passes through the mirror when the angle of incidence is equal to that angle of incidence which would usually be encountered when using unmodified objective lens assemblies with such microscopy equipment. One type of such wavelength selective mirror is a dichroic mirror, which is commonly available where the angle of incidence is 45 degrees. Light in the spectrum below .750 micrometers (e.g. visible) passes through the mirrors toward the eyepiece 14 and to a still or video camera C.

In another embodiment the laser beam is of a wavelength in the ultraviolet region of the light spectrum. In this case a wavelength-selective mirror (e.g. mirror 20) reflects the laser wavelength toward the sample and stage, while the path of visible light is not affected.

In one preferred embodiment as shown in Figs. 2A and 2B an objective lens assembly 50 has an objective lens 52 in a barrel body 54. The barrel body 54 is designed to mate in both circumference and thread-type with commonly available microscopes. The barrel body 54 has a bottom 55, a top 56, and a side port 57. A laser input cylinder 58 is secured to the side port 57 (e.g. threadedly or with a lock ring). A laser beam or beams (as from a laser 30, Fig. 1) is input at an end 59 of the laser input cylinder 58. A wavelength-specific barrier filter 60 in the bottom 55 of the barrel body 54 prevents laser light from propagating in an undesired direction, typically toward the path of visible light (i.e., in the direction toward the eyepiece or the camera). A dichroic mirror 62 is mounted in an end 64 of the laser input cylinder 58 to direct laser light from the input at the end 59 of the laser input cylinder 58 in Fig. 2b toward a focusing lens unit 66. The focusing lens unit 66 is, preferably, an interchangeable lens unit. In one aspect it contains a lens 52 and is releasably secured to the barrel body 54. It may contain a combination of lenses or a lens system with multiple components. The lens 52 may be referred to as a frontal lens that focuses the laser light onto a target sample (e.g., sample S) and also collects a microscopic image from the sample. The lens 52 may have any desired optical characteristics. Laser blocking filters may be used to prevent laser light from reaching the microscope eyepieces or camera(s); e.g.

added to the objective lens assembly, to the camera mount, or to the eyepiece assembly.

In one embodiment an objective lens assembly 50 is mounted in place of the lens 16 (Fig.1) onto the microscope base 12. The position of the objective lens assembly 50 may be adjusted along an imaginary line parallel to the longitudinal axis of the barrel body 54 and orthogonal to the surface of the sample mounting stage 24. Via this linear adjustment of the objective lens assembly 50, the distance between the frontal lens 52 and a target sample placed on the stage 24 is controllable. This adjustment also accommodates various focusing needs at appropriate wavelengths to be projected through the objective lens assembly (e.g. ultraviolet, visible, and infrared wavelengths).

In one embodiment, an infrared laser source (e.g. laser light with a wavelength greater than 1200 nanometers is incorporated into the laser input cylinder 58 of the objective lens assembly 50. A small laser diode (shown optionally and schematically as item 70, Fig. 2b) is secured within the input cylinder 58, e.g. threadedly, and the emission of laser light from the diode is collimated through a collimating lens (shown optionally and schematically as item 69, Fig. 2b). The laser beam is then reflected by a wavelength-selective mirror 62 which directs the laser beam towards the frontal lens 52 and is thus focused onto the target sample. The wavelength-specific barrier filter 60 prevents the exit of light from the laser source from the barrel body 54. The wavelength-specific mirror 62 and the barrier filter 60 do not affect the path of other laser sources (e.g. wavelengths less than 1200 nanometers) nor the path of visible light through the microscope. Consequently, laser light may also be collected from other laser sources which are introduced through ports 34, 35, 36 and through

a series of lenses and/or mirrors, such as the mirrors 20, 22, 23 which are mounted onto and/or within the microscope base 12.

The present invention, in certain embodiments, discloses a laser beam steering mechanism for use in laser-assisted microscopy applications. In one aspect such a mechanism provides accurate alignment and positioning of a laser beam relative to the optical path of a microscope objective lens and includes a combination of lenses and mirrors mounted on independent precision micropositioners. Precise control over the relative positions of key optical elements of the system is provided. The mechanism can either be manually commanded or motorised for remote control operations. With this system, fine three-dimensional positioning of the laser beam focus spot within the viewing field of the objective lens is achieved independently from the image focal plane. The system is designed to accommodate both continuous wave and pulsed laser sources. Furthermore, optical configurations of the device are optimised for use over a broad range of wavelengths from the ultraviolet to the near infrared region of the electromagnetic spectrum. System design retains flexibility so that different lens combinations can be substituted to meet specific optical requirements from various objective lenses. Such a mechanism provides high precision and reliability in the optimisation of the alignment of laser beams used in microscopy and are useful, e.g. in the field in vitro laser-assisted measurement and analysis (spectrometry, energy transmission, molecule excitation).

Lasers (Light Amplification by Stimulated Emission of Radiation) generate coherent and distinct electromagnetic radiation in the ultraviolet (UV), visible, or infrared (IR) range of the spectrum. Lasers

provide very reliable and precise sources of light that can be used together with a microscope to perform various applications at the microscopic level. For example, laser beams associated with a microscope are used to manipulate, dissect or analyse microscopic specimens in vitro such as living cells, organelles, dust particles, and crystals. Different lasers provide various sources of light centred on the specific wavelengths that are appropriate for each distinctive application. Typically, lasers that emit in the IR range of the spectrum are used to capture and manipulate particles in a light gradient force called an "optical trap". Lasers that emit in the UV, in the visible, or in the IR range of the spectrum are used to perform microdissections that are referred to as "optical microdissection". Additionally, a variety of laser radiation, from the UV to the IR, are used to perform molecular analysis, activate physiological properties in biological samples, release caged molecules, excite compounds such as fluorochromes, and asses spectral properties from individual microscopic targets.

In certain applications, the successful use of laser beams in microscopy is contingent upon how well an investigator can efficiently control the beam parameters. In this respect, it is particularly important to manage laser beam intensity, wavelength and precise direction of propagation. Control over beam intensity is usually addressed by regulating the input power supply of the laser module or with the addition of neutral density filters in the beam path. Control over wavelength is afforded with a choice of specific laser modules; monochromatic radiation can also be obtained by introducing specific optical filters or dyes in the beam path. Control over direction of propagation is very delicate to handle and may require the use of a precise

optical fixture. In order to position and to focus the beam of light onto the target sample with a high level of precision, it is essential to accommodate the optical fixture with a minimum number of degrees of freedom. The fixture should permit a collimated beam to be introduced into the optical path of the microscope and to obtain a sharp spot at the focus point for the laser. To achieve this, accurate control needs to be maintained over both the alignment and the positioning of the laser beam relative to the objective lens that is used to focus the laser. Sub-optimal alignment of the laser may result in poor quality or inefficient optical trapping or optical microdissection and in inaccurate laser-assisted analysis. Control over laser beam propagation in microscopy applications is a difficult task to achieve and an appropriate optical fixture has not yet been disclosed or suggested in the prior art.

A mechanism and a procedure according to the present invention provide precise and reliable alignment and positioning of a laser beam relative to the optical path of a microscope and of an objective lens; and such a mechanism provides accurate adjustments for the laser in three dimensions within the field of view of the objective lens, but independently from the image focus plane. Such a fixture in one aspect has an assemblage of optimised lenses and mirrors that can be accurately positioned relative to each others. The mechanism forms an interface between a laser module and a microscope or microscope objective lens. The overall design for the optical fixture and its principle of operation remain common to the various fields for which it finds applications, such as optical trapping, optical microdissection and laser-assisted molecular studies.

A convenient way to apply a beam of light to a microscopic sample is to introduce the laser within the

optical path of a microscope's objective lens. This method is referred to as the "non-contact mode" because no mechanical device touches the specimen during light irradiation and only the focused beam of light is applied to the sample. To deliver the beam of photons to the target sample, the laser beam is focused through the same objective lens of the microscope that is used to view the specimen. The non-contact mode of light irradiation is currently applied to the formation of optical traps and for performing laser microdissections. Also, many laser-assisted molecular studies are based on this mode of operation. However, it must be emphasised that the non-contact mode of light distribution in microscopy is only efficient in a limited range of wavelengths, typically from 200 nm to 2000 nm (a range of wavelengths that covers most biological applications). This limitation results from properties of light propagation through glass and through water. On one hand, only exotic glass materials are transparent to wavelengths less that 200 nm. On the other hand, light in excess of 2000 nm does not travel very far in aqueous medium as a result of increased absorption from water molecules. In aqueous medium, wavelengths in excess of 2000 nm must be delivered to a sample through an optical fibre. ("nm" is nanometers.)

Optical trapping refers to the phenomenon that allows the capture, with a cage of light, of a microscopic particle in suspension. This phenomenon results from the fact that refraction is the dominant form of light scattering for transparent particles of diameter in the micrometer range. The refraction of the photon in the incident source of light as they pass through the particle causes a change in their momenta. Some of the momentum is imparted to the particle and causes it to move. An optical trap is obtained using a

laser beam as the source of incident light and a large numerical aperture objective lens to sharply focus the beam. The distribution of photons in the trapping laser beam is typically Gaussian. The gradient of light reaches a maximum at the centre of the beam and the photon density (photon flux or light intensity) gradually diminishes towards the periphery. Focused using a large numerical aperture objective lens, the laser beam converges into a spot of greatest light intensity at the focus point for the laser. This spot defines the optical trap. A particle exposed to the focused beam encounters a greater number of photons closer to the beam centre and closer to its focus point. If such a particle has a higher refractive index than its surrounding medium, then the net reaction force that results from the change in photon momenta, is directed toward the trap. It is at this point that the particle reaches a position of stability, as the forces acting on it are in equilibrium.

When forming an optical trap, it is important to chose a wavelength that is not absorbed nor reflected by the particle. Absorption of the incident light would result in the destruction of the target particle following heat elevation(particularly in the case of biological samples). Reflection of the incident light would result in unbalanced forces and instability of the particle in the optical trap. Optical traps that are non-traumatic for most samples are obtained using an infrared laser beam(typically from 750 nm to 1100 nm). Although an optical trap can be formed at other wavelengths (e.g. in the visible range), IR wavelengths are preferred because most biological samples as well as water absorb very little energy in this region of the spectrum. Consequently, most specimens such as cells, organelles, chromosomes or other microscopic particles

such a liposomes and microspheres can be held in a 750 nm - 1100 nm trap without being damaged.

Optical microdissection is based on the principle of laser ablation. The ablation process results from phase changes of liquid and solid components. One major phenomenon that occurs during optical microdissection is the ablation of the target specimen as a result of elevation of heat as laser light is absorbed by the specimen. This phenomenon is strongly influenced by the absorption properties of the target sample, as well as by the characteristics of the laser beam. The efficiency of the laser beam in optical microdissection applications is measured in terms of penetration depth. The penetration depth of the laser beam is dependent in part upon how well the laser beam can be focused on the target, but is also a function of the available light intensity and of its wavelength. Absorption of the laser light in the sample is commanded by the potential presence of different chromophores (its spectral absorption properties).

Laser thermal ablation applications have been described using various lasers, some of which produce wavelengths that are better transmitted by microscope optics and therefore are more appropriate for the non-contact mode of sample irradiation. Wavelengths in the UV (e.g. 308 nm, 337 nm, 355 nm, 390 nm) or in the visible range (450 nm, 590 nm, 640 nm, etc. ...) are used for microdissection of biological samples based on the absorption properties of some of their molecular constituents such as proteins. In the IR range of the spectrum (i.e. 1480 nm) the ablation phenomenon is based for a large part on the absorption property of water molecules and the microdissection of a specimen results from the local elevation of temperature in the surrounding aqueous medium or constitutive water

molecules. The ablation phenomenon, at these wavelengths, may also result from biochemical and structural transformations induced in the target specimen. In such a case, the photolysis phenomenon depends on the structural organisation of the target sample more than on its absorption characteristics.

Lasers are useful tools to perform a number of molecular studies and analysis in vitro. They are preferred over standard illumination bulbs for their beam characteristics that constitute a more amenable source of light. Various wavelengths of light are used to trigger photodynamic physiology reactions in live biological specimens that possess specific pigments or receptors sensitive to the emitted wavelength. Using a laser beam to deliver the incident source of light provides accuracy and precision in the excitation process. Light is also useful to activate biologically active molecules or "cage compounds". These biologically active molecules are enclosed in a photo-labile "molecular cage" and are intended to be introduced into live cells by microinjection or after permeabilisation of the cell membrane. The chemical bound between the cage and the biologically active molecule is then released by irradiation with pulses of UV light. The associated physiological responses are then recorded according to time. In order to permit the molecular activation in specific locations only within the viewing field of the microscope objective lens, such as surrounding or inside a target specimen, a laser is preferred as source of light because it can be directed with precision and focused tightly within the sample. Light is used to trigger the primary autofluorescent properties of molecular structures that are naturally present in a given specimen as well as synthetic fluorochromes associated to highly specific molecular probes.

Monochromatic, collimated laser beams are advantageous for such an application. The detection of emission fluorescence signals provides information about the spectral properties from individual microscopic targets.

Procedures are known for optical trapping, optical microdissection and laser-assisted molecular studies. Focused laser beams associated with laboratory microscopes are known and have been used as a tool and an efficient approach for the non-contact micromanipulation or microanalysis of microscopic specimens in vitro. From a technical perspective however, the successful application of lasers to microscopy depends on the interface of these two devices. A stable optical trap cannot be obtained if the incident laser beam is not rigorously parallel to the optical path of the objective lens. Any significant angle in the incident beam of light would result into imbalance in the net force applied to the particle that results from momentum transfer and consecutively into instability of the particle within the trap. Similarly, instability of the particle in the trap would also occur if the back aperture of the objective lens is not properly filled with an homogeneous density of light. The use of a laser beam to perform optical microdissection applications responds to similar concerns. A sharp and intense laser focus spot, as is necessary to perform submicrometer sized incisions, requires a laser beam that is well positioned and aligned relative to the objective lens (axis of the laser beam centred on and parallel to the axis of the optical path of the lens). Laser-assisted microscopy, according to this invention, makes it is possible to place and stir easily and independently each one of the laser beams anywhere in the field of view of the microscope objective lens. This is particularly important when optical trapping, optical microdissection

or laser-assisted molecular studies need to be performed in multiple and various positions of a give specimen.

These considerations emphasise how important precise controls over laser beam characteristics are in order to achieve properly the applications mentioned above. Controlling the angle of divergence as well as the direction of propagation of the incident laser beams is critical. Also critical is controlling the position of the focus spot of the laser beam in the three dimensions, as defined by the objective lens. It is therefore very significant to interface a given laser module together with a microscope or with a microscope objective lens using the most appropriate control mechanism. Such a control mechanism according to the present invention achieves three dimensional laser beam alignment and positioning relative to the optical path of the microscope and of the objective lens. Without such a device, it would be very difficult to perform, with accuracy, the laser-assisted microscopy procedures mentioned earlier (optical trapping, optical microdissection, molecular studies). A number of mechanical and optical features are implemented in the control mechanism in order to address specific technical objectives.

The present invention discloses, in certain embodiments, a mechanism including lenses, mirrors and associated articulations that achieve the alignment and positioning of a laser beam relative to the optical path of a microscope or a microscope objective lens. Such a mechanism is useful for applications in the non-contact mode of laser assisted microscopy and with both continuous wave as well as pulsed laser sources. Optical elements of the device are optimised for selected wavelengths from the UV to the IR range (typically from 200 nm to 2000 nm). In one aspect such a mechanism

provides simplicity and accuracy in the positioning of a laser beam relative to the optical path of the microscope and/or objective lens and provides simplicity and accuracy in the alignment of the laser beam relative to the optical path of the microscope and/or objective lens. Articulation of key optical elements of the mechanism provides control over the spatial characteristics of the laser beam such as divergence/convergence angle and propagation angle and control settings of such mechanism permitting the rapid interfacing of a laser beam with various objective lenses of varying back aperture sizes. Such mechanism provides precise control over laser focus spot positioning in both the X and Y axis of the objective lens viewing field and precise control over laser focus spot positioning in the Z axis independently from the image focal plane (parfocality adjustment). Such a mechanism may be compact and compatible with the dimensions of standard microscopes and, in one aspect, is easily manufactured. Such a mechanism may be manually controlled, or it may be motorised for remote control operations.

Several laser options are possible with certain embodiments of a mechanism according to the present invention. These laser options cover a wide range of wavelengths, from the UV to the IR of the electromagnetic spectrum. In certain embodiments, the range of wavelengths is from 200 nm to 2000 nm. Optical elements including lenses and mirrors are respectively selected for optimum transmission ratio or reflection characteristics at specific wavelengths or wavelength ranges. For applications in the IR, mirrors are preferably gold coated for optimum IR reflection. Such applications include optical trapping (typically from about 750 nm to 1100 nm) and optical microdissection (typically from 1200 nm to 2000 nm in the non-contact

mode). Enhanced aluminium mirrors are, preferably, employed when working in the visible range and reflectors UV corrected are preferred for working from 200 nm to 400 nm (optical microdissection and molecular analysis applications).

It is desirable to provide control over laser beam positioning relative to the optical path of a microscope and/or of an objective lens. Such laser beam positioning superimposes the centre of the laser beam to the centre of the optical path of the objective lens. A mechanism according to the present invention provides an operator with controls to achieve such laser beam positioning.

In the Figs. 3 - 9, items are numbered as follows: laser beam positioning component 110, circular wedge prism 111, circular wedge prism 112, rotary positioning mounting stage 113, rotary positioning mounting stage 114, first wedge apex angle 115, second wedge apex angle 116, wedges' rotation axis 117, laser beam 118, wedges' adjacent faces 119, laser beam alignment component 120, mirror 121, mirror 122, articulated angle mount 123, stationary angle mounting surface 124, adjustment screw 125, adjustment screw 126, parfocality adjustment component 130, Keplerian telescope stationary output lens 131, Keplerian telescope movable lens 132, precision miniature stage positioning mechanism 133, precision rack 134, pinion slide 135, knob 136, objective lens 137, back aperture of the objective lens 138, laser focus spot 139, image focal plane of the objective lens 140, X-Y laser control component 150, adjustable tilt table 151, thumb screw 152, thumb screw 153, stationary mounting frame 154, pivot point 155.

In one aspect control over of the incident angle of a laser beam is achieved by using a pair of circular wedge prisms 111 and 112 used as beam steering elements. A laser beam positioning component 110 is shown in Figure

3. The deviation of a laser beam 118 passing through the two wedges 111, 112 is determined by apex angles 115 and 116 of the wedge prisms 111, 112 which are, preferably, of equal power (equal deviation) positioned in near contact. The wedge prisms can be independently rotated using rotary positioning mounting stages 113 and 114 with adjacent faces 119 parallel to each other and perpendicular to their rotation axis 117. A laser beam 118 passing through the combination of the wedge prisms 111, 112 is steered in any direction. This association of a pair of wedge prisms constitutes one component 110 that permits the control of subtle deviation angles of an incident laser beam. Such subtle deviation angles are circumscribed within a narrow cone, that is typically within the path of the undeviated incident beam. This permits very precise super-imposition of the centre of the laser beam to a centre 127 of an optical path of an objective lens 137 (as shown on Fig. 3). The wedge prisms 111, 112 are preferably of material optimised for specific wavelengths of various incident laser beams (e.g. manufactured in synthetic fused silica for UV applications or combined with various antireflection coatings).

Control over laser beam alignment relative to the optical path of a microscope and/or an objective lens is provided by the present invention. The purpose of laser beam alignment is to obtain parallelism between the axis of the laser beam and the axis of the optical path of the objective lens.

Control over alignment of the incident laser beam is achieved, in one aspect, using a pair of quasi-parallel mirrors 121, 122 used as beam steering elements. A laser beam alignment component 120 is shown in Fig. 4. An angle of deviation of the laser beam 118 reflecting on the two mirrors is controlled by displacing independently

one mirror relative to the other. A first mirror 121 is held by an articulated angle mount 123 which is fitted with fine resolution, adjustment screws 125 and 126 for precise tilting of the mounting surface in two directions normal to each other. The second mirror 122 is fixed to a stationary mounting surface 124. This component permits precise control of the incident angle of the laser beam relative to the optical path of the objective lens. This approach permits the precise alignment of the axis of the laser beam 118 parallel to axis of the optical path 127 of the objective lens 127 as shown in Fig. 5. The mirrors are selected in material optimised for specific wavelengths of various incident laser beams (e.g. manufactured in specific materials or combined with various reflection coatings).

Control over the positioning of the focused laser spot along the Z axis independently from the image focal plane of a microscope is referred to as "parfocality adjustment". This feature allows an investigator to displace the focus spot for the laser beam in the Z direction without having to move the objective lens. This operation permits an operator to either place the laser focus spot within the same focal plane as the image focal plane, or above or below.

Control over laser beam positioning along the Z axis (parfocality adjustment) is achieved using a telescope used as a beam expander. In one aspect, either a Keplerian telescope or a Gaussian telescope is used. The Keplerian telescope uses two positive lenses while the Gaussian telescope includes a combination of one negative lens with one positive lens. The choice of a style of telescope requires minor modifications in the positioning of the optical components of the system relative to each other. The Keplerian telescope (Fig. 5) has an optical configuration that offers more room for the association

of complementary optical elements. A parfocality adjustment component 130 is shown in Fig. 5. The telescope beam expander consists of two lenses. A first lens 131, positioned on the side of the telescope that faces a back aperture 138 of the objective lens 137, is stationary. A second lens 132, positioned on the side of the telescope that faces an incident laser beam 118, is movable along the same axis as the incident laser beam axis. Precision linear displacement of this lens is achieved using a precision miniature stage positioning mechanism 133. The positioning stage includes a precision rack 134 and a pinion slide 135 driven by a knob 136.

Fig. 6 illustrates the operation of the telescope relative to an axis 127 of the optical path of an objective lens 137. The axis of the laser beam 118 is aligned parallel and co-axial to the axis 127 of the optical path of the objective lens 137. In Fig. 6 only a schematic representation of the objective lens 137 is depicted. The diameter of the incident laser beam is determined by the characteristics of the laser; this beam is predominantly collimated but may display a slight divergence angle due to the characteristics of the laser itself.

With the lenses 131 and 132 positioned in a reference position (Fig. 6), the laser beam 118 at the exit of the telescope is still collimated. The diameter of the beam is then determined by the ratio of the two lenses (as a typical example, with an incident laser beam of a 2.5 mm diameter, the beam diameter is expended to 8.0 mm by the telescope). With the lenses of the telescope positioned in the reference position, the focus spot of the laser 139 is placed on the same focal plane as the image focal plane 140 of the objective lens 137.

When the movable lens 132 is displaced further away from the stationary output lens 131 (Fig. 6), the laser beam 118 is converging at the exit of the telescope. However, since this beam is also larger in diameter at the level of the output lens 131, it reaches the same diameter as the diameter of the collimated reference beam beyond the end of the telescope at a distance equal to the focal length of the stationary output lens. In this configuration, the focus spot 139 for the laser beam is closer to the front of the objective lens 137 than with the reference collimated beam. The laser focus spot 139 is positioned between the front of the objective lens 137 and its image focal plane 140.

When the movable lens 132 is displaced closer to the stationary output lens 131 (Fig. 6), the laser beam 118 is diverging at the exit of the telescope. However, since this beam is also smaller in diameter at the level of the output lens 131, it reaches the same diameter as the diameter of the reference collimated beam at a distance equal to the focal length of the stationary output lens. In this configuration, the focus spot 139 for the laser beam is further away from the front of the objective lens 137 than with the reference collimated beam. The laser focus spot 139 is positioned beyond the image focal plane 140 of the objective lens 137.

In one aspect the stationary output lens 131 of the telescope is placed at a distance equal to its focal length from the back focal plane of the microscope objective lens 137 which is located at its back aperture 138 (as seen in Fig. 9). Therefore, the diameter of the laser beam is maintained constant at the back focal plane of the objective lens regardless of the position of the movable lens 132. The diameter of the laser beam 118 at the objective is selected to be slightly larger than the back aperture of the objective lens 138 for optimum

performance in laser-assisted microscopy applications. It should also be noted that the focal plane of an objective lens varies with wavelengths. Due to this chromatic aberration, the focal plane of an objective lens is somewhat different in the UV, visible and IR range of the spectrum. The parfocality adjustment described above permits compensation for this phenomenon.

Adjusting the diameter of the laser beam relative to the size of the back aperture of the objective lens helps control the density of light that can be uniformly introduced within the back focal plane of the objective lens. This feature accommodates different objective lenses with a varying width of back aperture. Also, controlling beam diameter at the back aperture of the objective lens accommodates for various laser beam divergence angles.

Control over laser beam diameter at the back aperture 138 of the objective lens 137 is achieved by using lenses of varying focal length in place of the movable lens 132 in the telescope beam expander as shown in Fig. 5. Various lenses at this position play the role of various beam expanders. These various lenses are held on the path of the incident laser beam by a precision miniature stage positioning mechanism 133. This miniature stage positioning mechanism includes a precision rack 134, a pinion slide 135, and a knob 136.

Control over fine positioning of the focused laser spot within the viewing field of the objective lens allows an investigator to focus the laser beam on any particular part of the observed specimen. An X-Y laser control component 150 provides such control.

The mechanism component used for laser beam positioning along X and Y axis of the viewing field is depicted in Fig. 7. Control over positioning of the focused laser beam within the viewing field of the

objective lens is achieved using, as beam steering element, the same pair of quasi-parallel mirrors used for laser beam alignment described in Fig. 4, but with another control mechanism. The angle of deviation of the laser beam 118 reflecting on the two mirrors is controlled by tilting simultaneously both mirrors 121 and 122 around a pivot point 155. The two quasi-parallel mirrors are rigidly mounted on an adjustable tilt table 151 which is fitted with fine resolution, adjustment thumb screws 152 and 153 held in a stationary mounting frame 154, for precise orientation of the two mirrors relative to the axis of the incident laser beam 118. Orientation of the two mirrors is in two directions normal to each other.

The operation for laser beam positioning along X and Y is shown in Fig. 8. Control over X-Y positioning of the focused laser spot 139 within the viewing field of the objective lens is implemented by de-centring the incident laser beam 118 from the stationary output lens 131 of the telescope beam expander component. In this configuration, the laser beam remains collimated at the exit of the telescope (defined by the lenses 131), but exits from it at an angle. The axis of the laser beam 118 crosses the axis 127 of the optical path of the microscope objective lens 137 at a point equal to the focal length of the stationary lens 131 from the end of the telescope. The diameter of the laser beam 118 remains the same at the level of the back aperture 138 of the objective lens. Therefore, the density of light introduced at the back focal plane of the objective lens by the de-centred laser beam remains identical as the density of light that would be introduced at the back focal plane of the same objective lens by a non de-centred laser beam. As a result of de-centring the laser

beam 118, the laser focus spot 139 is proportionally displaced within the image focal plane 140.

As an operating system, the mechanism described in the present invention offers an operator the controls useful for achieving precise laser-assisted microscopy applications. These controls are intended for a) superimposing the centre of the laser beam to the centre of the optical path of the objective lens, b) for obtaining parallelism between the axis of the laser beam and the axis of the optical path of the objective lens, c) for achieving parfocality adjustment, d) for adjusting the laser beam diameter at the back aperture of the objective lens, and e) for X-Y positioning of the focused laser spot within the field of view of the objective lens.

The mechanism described includes the association of the various components described above. The operating system is shown in Fig. 9. It includes the laser beam positioning component 110, the laser beam alignment component 120, the parfocality adjustment component 130 that also serves for adjusting the laser beam diameter at the back aperture of the objective lens, and the X-Y laser control component 150. The laser beam positioning component is placed first on the path of the incident laser beam. The laser beam alignment component as well as the X-Y laser control component are both placed within the boundaries of the Keplerian telescope beam expander that serves as parfocality adjust component. These components contribute to providing control over positioning of the laser focus spot 139 in the three dimensions relative to the image focal plane 140 of the objective lens 137.

Whilst the use of an eyepiece has been described in connection with the preferred embodiments, any suitable form of viewing means could be used, for example an eyepiece, CCD camera, video camera, spectrometer,

photographic camera or any means which records any wavelength or range of wavelengths of the electromagnetic spectrum.

In the claims that follow 'to manipulate' a sample on a stage of a microscope includes, but is not limited to, moving, ablating, and/or cutting the sample.

CLAIMS:

1. A microscope comprising

a hollow base,

viewing means,

an objective lens assembly,

a stage for holding a sample to be studied by the microscope,

and at least one port in the base through which at least one laser beam is directable to manipulate a sample positioned on the stage.

2. The microscope of Claim 1 further comprising at least one laser for providing the at least one laser beam.

3. The microscope of Claim 2 wherein the at least one laser is mounted exterior to the base of the microscope.

4. The microscope of Claim 2 wherein the at least one laser is mounted within the base.

5. The microscope of Claim 2, 3 or 4 wherein the at least one laser is a diode laser.

6. The microscope of Claim 2, 3, 4, or 5 wherein the laser beam is from the group consisting of ultraviolet, visible and infrared.

7. The microscope of any preceding Claim wherein the at least one laser is a plurality of lasers.

8. The microscope of any preceding Claim wherein the objective lens assembly is an interchangeable assembly.

9. The microscope of Claim 8 wherein the interchangeable lens assembly has at least one laser beam port.

10. The microscope of Claim 8 or 9 wherein the interchangeable assembly has at least one laser mounted therein.

11. The microscope of any preceding Claim further comprising at least one wavelength selective mirror on which the at least one laser beam is directed.

12. The microscope of Claim 11 wherein the wavelength selective mirror permits simultaneous illumination of a sample on the stage and laser irradiation of the sample.

13. The microscope of Claim 12 wherein the wavelength selective mirror permits visual observation and/or photographic recording of the sample.

14. The microscope of Claim 10 wherein the at least one wavelength selective mirror is a plurality of wavelength selective mirrors which provide the simultaneous transmission and reflection of selected wavelengths of laser light

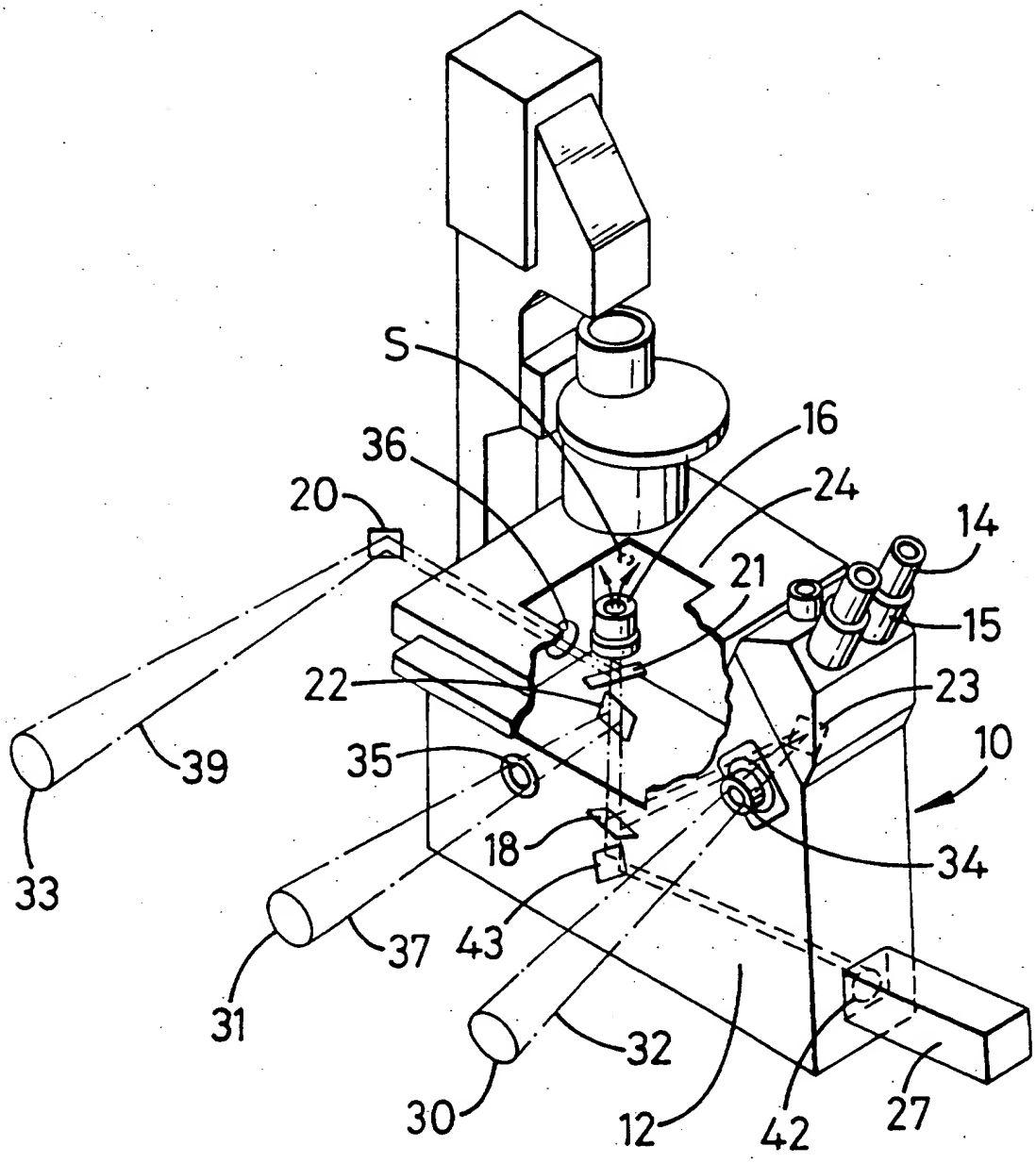
15. The microscope of any preceding Claim wherein the stage is movably connected to the hollow base.

16. The microscope of Claim 15 further comprising a motorised assembly connected to the stage for selectively and controllably moving the stage with respect to the base and with respect to the objective lens assembly.

17. The microscope of any preceding Claim further comprising at least one barrier filter for filtering the at least one laser beam to exclude light of selective wavelengths from the at least one laser beam.

18. The microscope of Claim 17 wherein the at least one laser is at least two lasers, the at least one laser beam is at least two laser beams, and the microscope further comprising a barrier filter for each of the at least two laser beams.

FIG. 1



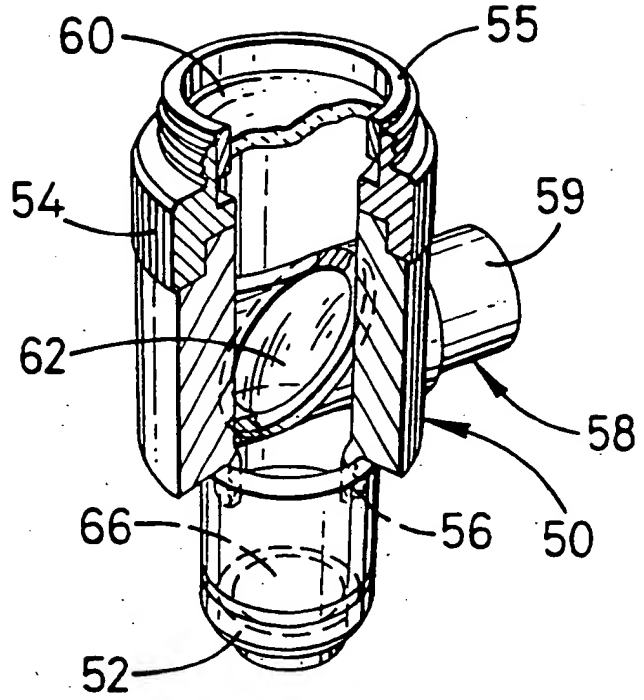


FIG. 2A

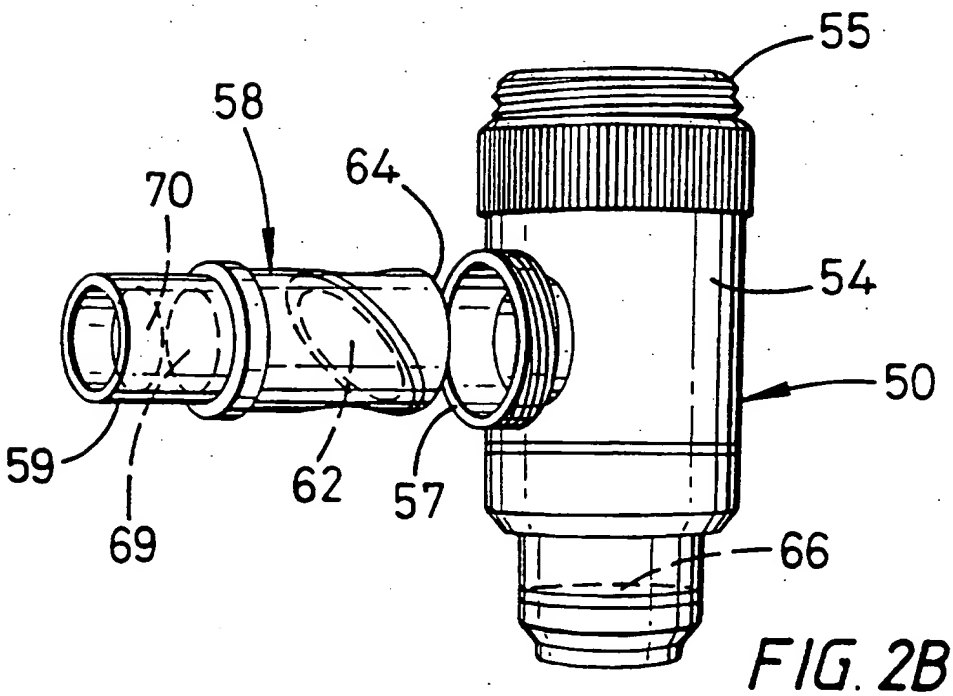
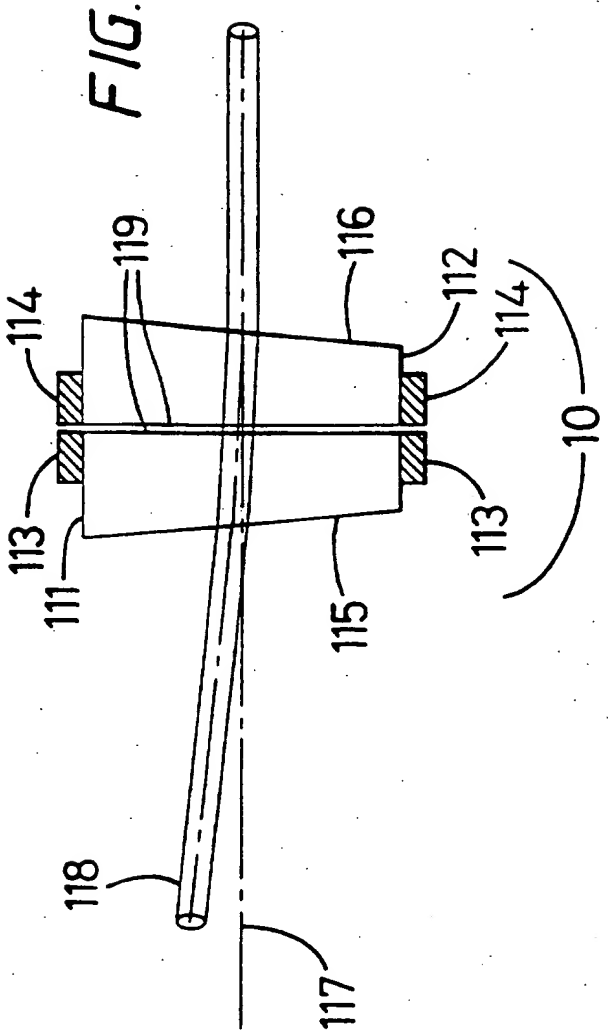


FIG. 2B

FIG.3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119
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112
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10
113
111
115
113
118
117

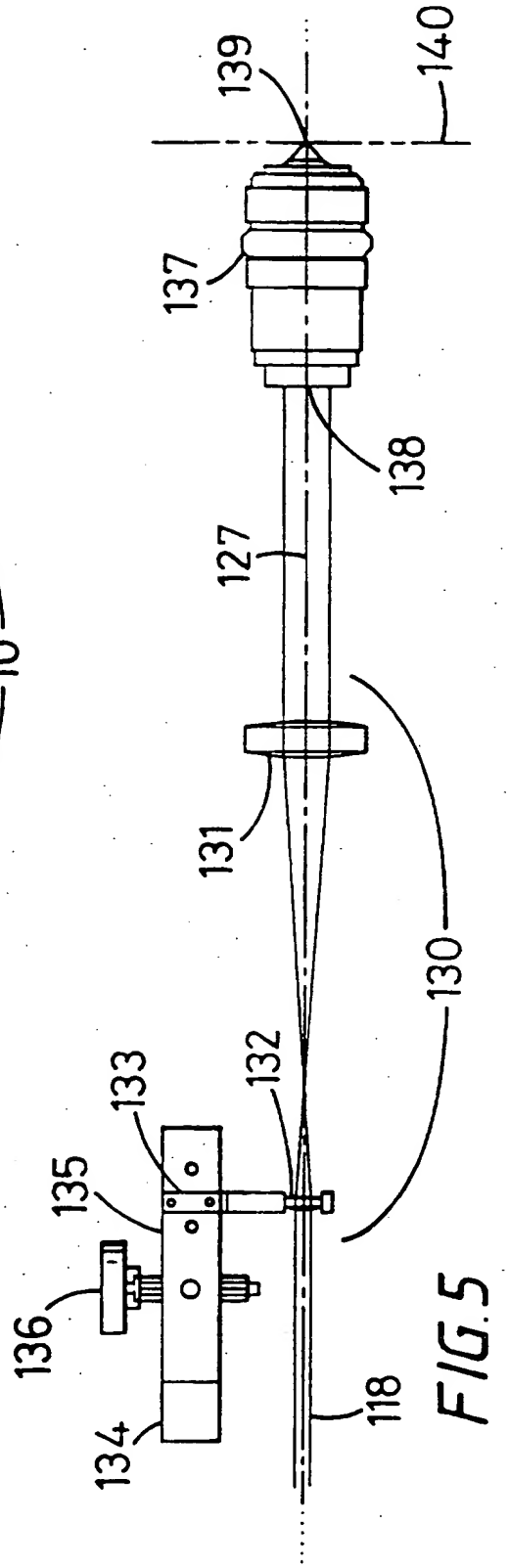
139
140
137
138
127
131
130
132
133
135
136
134
118

FIG.5

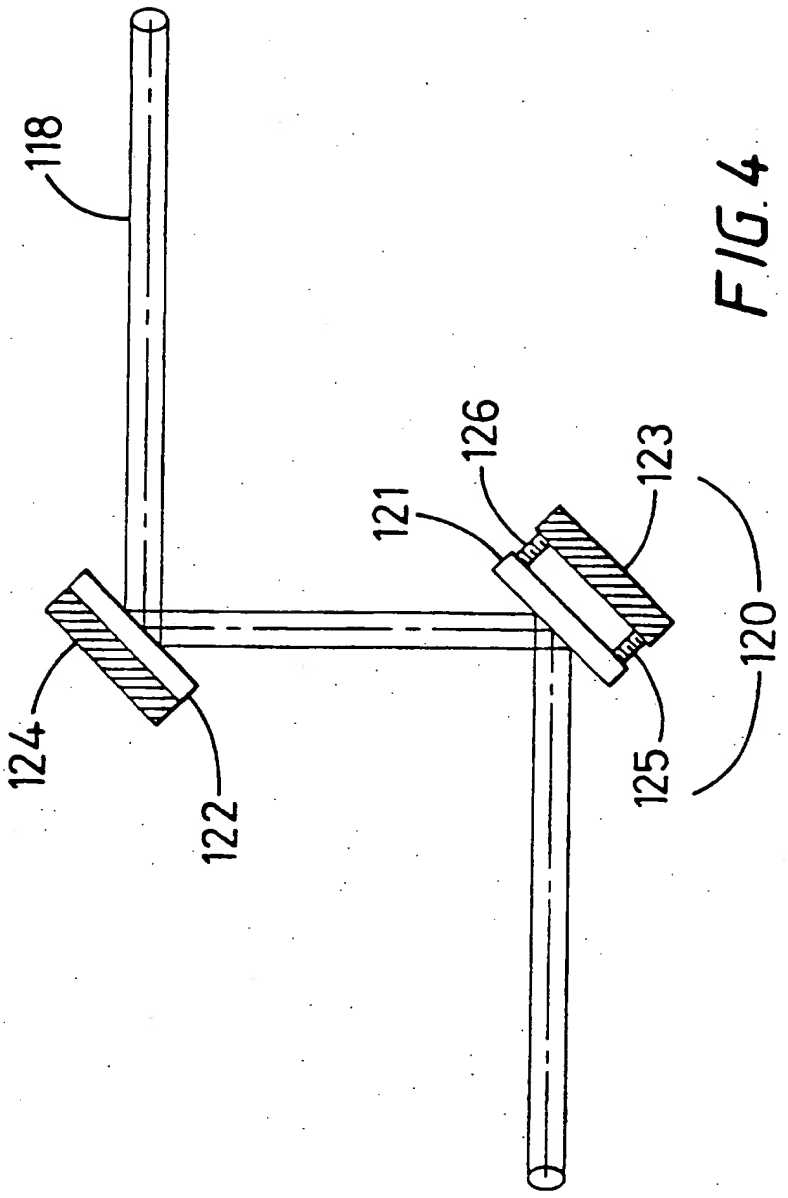

FIG. 4

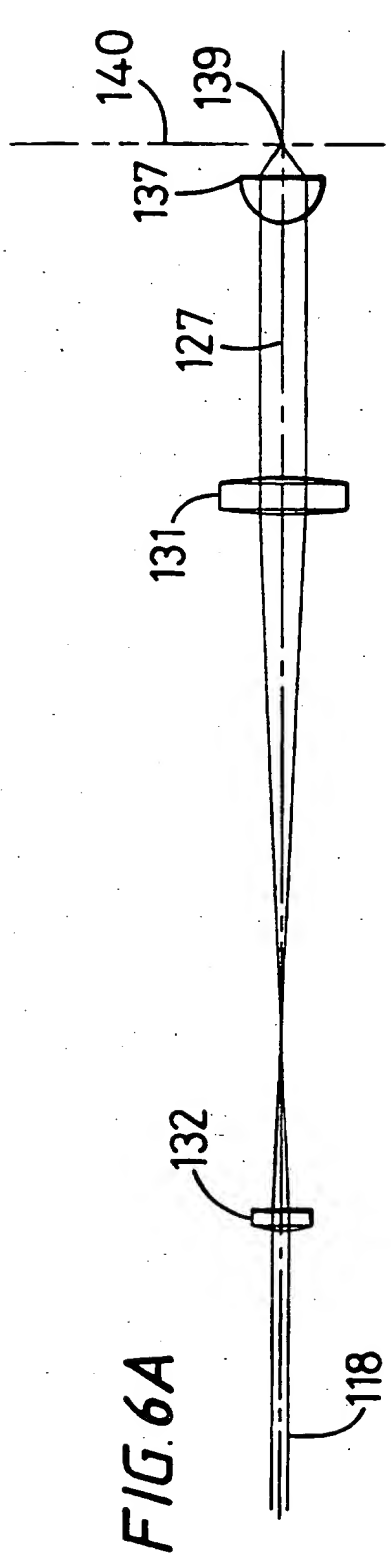

FIG.6A

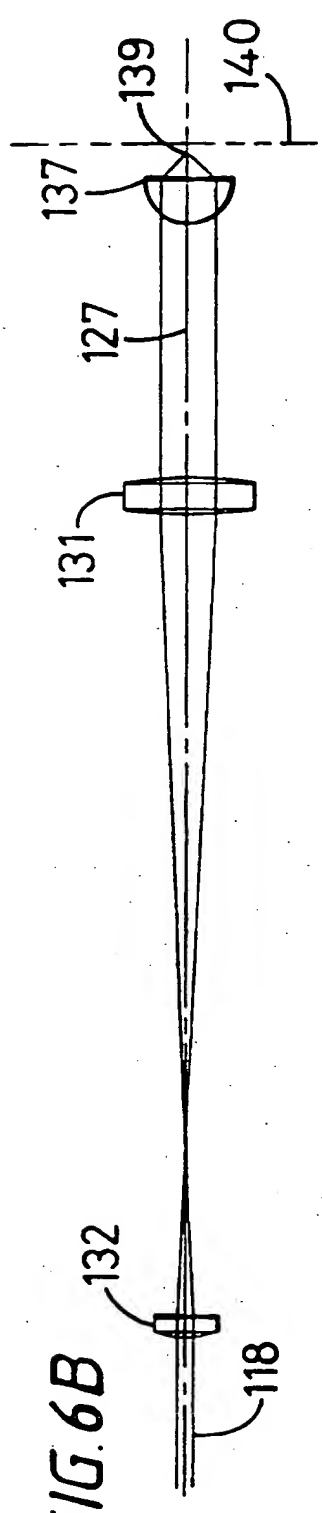

FIG.6B

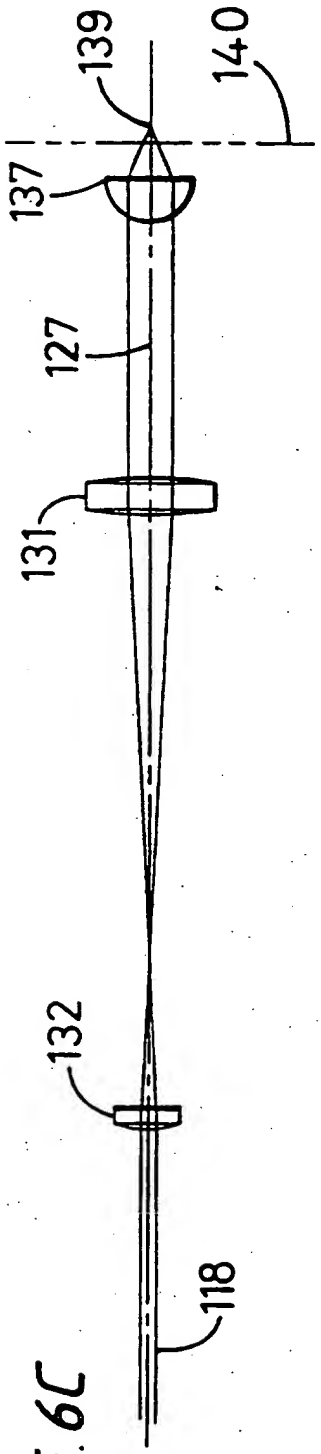

FIG.6C

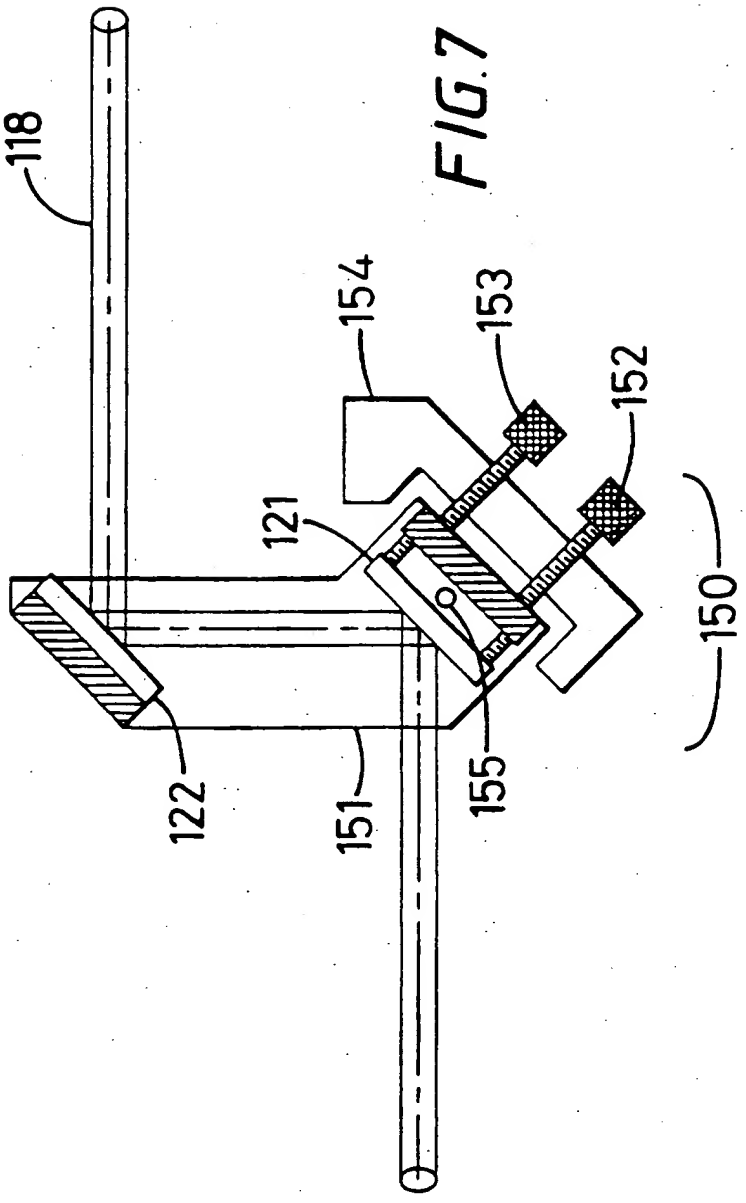

FIG.7

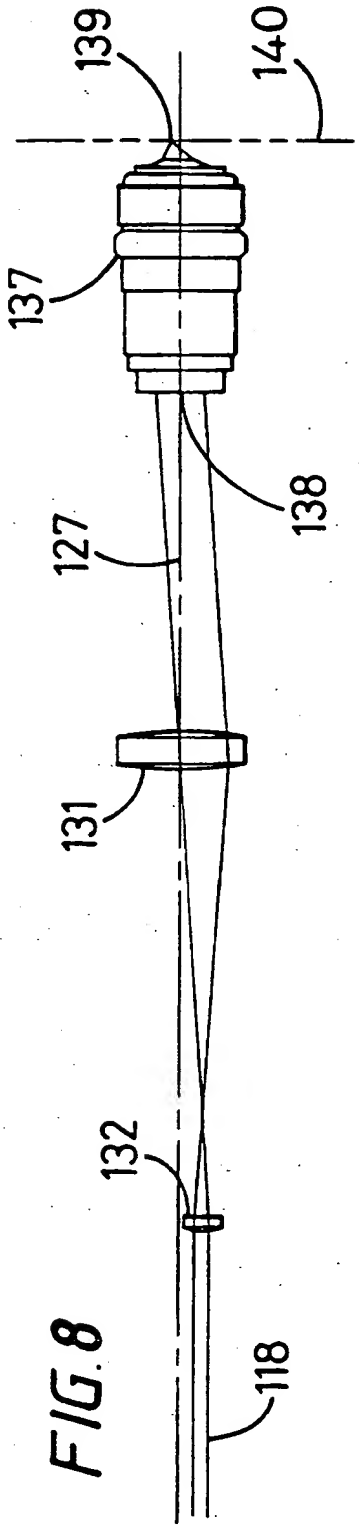

FIG.8

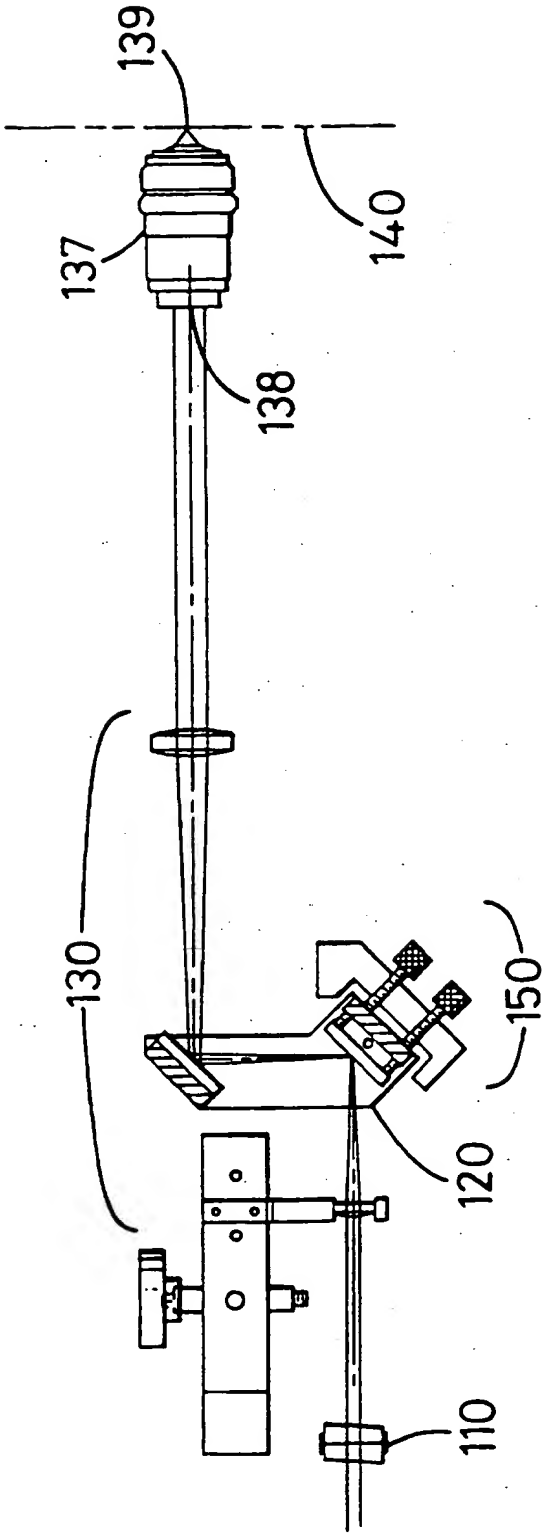

FIG. 9

INTERNATIONAL SEARCH REPORT

Intern ial Application No
PCT/GB 97/02681

A. CLASSIFICATION OF SUBJECT MATTER
IPC 6 G02B21/32 G02B21/00

According to International Patent Classification(IPC) or to both national classification and IPC

B. FIELDS SEARCHED

Minimum documentation searched (classification system followed by classification symbols)
IPC 6 G02B

Documentation searched other than minimumdocumentation to the extent that such documents are included in the fields searched

Electronic data base consulted during the international search (name of data base and, where practical, search terms used)

C. DOCUMENTS CONSIDERED TO BE RELEVANT

Category	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
X	US 4 629 687 A (SCHINDLER MELVIN S ET AL) 16 December 1986 see the whole document ---	1-3,6, 11,15-17
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A	see the whole document ---	18
X	EP 0 137 504 A (RIKAGAKU KENKYUSHO) 17 April 1985 see the whole document ---	1-3,6,7, 15,16
X	EP 0 501 688 A (HITACHI LTD) 2 September 1992 see the whole document ---	1-3,11, 15
	-/--	

[X] Further documents are listed in the continuation of box C.

[X] Patent family members are listed in annex.

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"P" document published prior to the international filing date but later than the priority date claimed

"T" later document published after the international filing date or priority date and not in conflict with the application but cited to understand the principle or theory underlying the invention

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"&" document member of the same patent family

Date of the actual completion of theinternational search	Date of mailing of the international search report
9 February 1998	19/02/1998

Name and mailing address of the ISA	Authorized officer
European Patent Office, P.B. 5818 Patentlaan 2 NL – 2280 HV Rijswijk Tel. (+31-70) 340-2040, Tx. 31 651 epo nl, Fax: (+31-70) 340-3016	Ward, S

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Intern ial Application No
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C.(Continuation) DOCUMENTS CONSIDERED TO BE RELEVANT

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X	US 5 363 190 A (INABA HUMIO ET AL) 8 November 1994 cited in the application see the whole document	1-3,6, 11,15,16
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